Cytogenetics of Tropical Bulbous Ornamentals

IV. Nature of triploidy in *Crinum augustum*

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The present cytological understanding of the genus *Crinum* is chiefly based on a study of its mitotic complements which may not give an exact idea about the innate cytogenetic constitution of its chromosomes. Both male and female meiosis take place when flowers are well within the bulb which have to be virtually sacrificed to extricate young anthers and even then one is not sure if the material contains appropriate meiotic stages. This has been an inherent limitation in building up a precise genetic-evolutionary picture of the genus. However, the few meiotic studies undertaken so far have revealed that the mitotic stability in *Crinum* (Raina and Khoshoo 1971a) cannot be used as an index to stability in innate structure and homology of its chromosomes (Khoshoo and Raina 1968). Similarly, the meiosis of triploid *C. augustum*, reported in this communication, has proved rewarding in assessing the nature of its triploidy. Furthermore, it also revealed that although this taxon is a mitotic mosaic (Raina and Khoshoo 1971b), only normal complements are selected in the male germ line. The methods used are the same as reported earlier (Khoshoo and Raina 1968).

Observations

Although the species is a chromosomal mosaic and shows reasonable amount of aneusomaty (Raina and Khoshoo 1970b) the present observations based on an analysis of 28 cells show conclusively that only cells with the full chromosome complement ($2n=33$) are selected in the germ line. Chromosome associations in pollen mother cells at metaphase I have been summarized in Table 1. As is evident on average there are $0.46_{III}+10.51_{II}$ and 10.6_I per

Table 1. Chromosome associations at metaphase I

Association	Trivalents	Bivalents	Univalents
Total number (28 cells)	13	294	297
Average number per cell	0.46 ± 0.09	10.51 ± 0.18	10.6 ± 0.23
Range per cell	0-2	8-13	7-13

cell. There is a very low trivalent frequency and the number never exceeds two per cell (Fig. 1). On the other hand, bivalents and univalents were

present approximately in equal numbers (Table 1). The most common (46.4%) association found was $11_{II}+11_I$ (Fig. 2). Univalents were always lying outside the metaphase plate. The chiasma frequency ranged from 30 to 40 per cell and the average per cell is 35 ± 0.5 . The average number of chiasma

Figs. 1-5. 1-2, metaphase I, $1_{III}+10_{II}+10_I$ and $11_{II}+11_I$. 3, anaphase I, 13:8:12. 4, telophase I showing lagging univalents and chromatids. 5, early telophase I with one bridge-fragment and precociously dividing univalents. $\times 1,000$.

terminalized is 9.2 and terminalization coefficient is 0.28. There is a positive correlation between the chromosome length and number of chiasmata as also postulated by Darlington (1965). The bivalents showed random distribution

of chiasmata as in *Tulipa*, *Chorthipus* (Darlington 1940) and species of *Allium* (Ved Brat 1965).

Anaphase I was characterized by unequal distribution of chromosomes to poles (Fig. 3). An analysis of 33 cells shows that the number at a pole may be 10-14, 17 or 21 (Table 2). The peak is around 11 and 12. The most

Table 2. Analysis of anaphase I in *C. augustum*

Chromosome distribution	Number of undivided and/or divided univalents in between the poles	Number of cells	Percentage
11:11	11	12	36.3
12:11	10	4	12.1
13:12	8	3	9.0
13:11	9	2	6.0
21:11	1	1	3.0
17:14	2	1	3.0
14:13	6	1	3.0
13:13	7	1	3.0
14:10	9	1	3.0
Not countable	10	4	12.1
"	8	2	6.0
"	13	1	3.0

common distribution found in 36.3% cells is 11:11 at each pole with 11 univalents dividing precociously in the middle. The number of undivided or divided univalents range from 1 to 13, the most common number is 11 (Figs. 4-6) and by the time telophase I sets in, the univalents complete division and form micronuclei. Average number of micronuclei per cell is 15.3. The micronuclei were also found at telophase II and in tetrads and pollen grains. Majority of the cells were devoid of bridges but several abnormalities were found both at anaphase I and II. There were 0-3 and 0-2 bridges with or without fragments at anaphase I and II respectively and one such configuration was invariably seen (Fig. 5). Another abnormality found in a very small percentage of cells is misdivision of univalents at anaphase I (Fig. 6) and chromatids at anaphase II (Fig. 7). The two arms, double at anaphase I and single at anaphase II, often move to the respective poles and form telocentrics, much like the monosomics of wheat (Sears 1952). Rarely some cells at anaphase II showed excessive fragmentation.

Pollen grain mitosis

In a triploid theoretically one can expect chromosome number in gametes ranging from n to $2n$ (Mather 1935). On this basis, of the 12 different chromosome numbers expected, only 7 were actually observed at anaphase I (Table 2). This was further tested by investigating pollen grain mitosis in order to find what numbers were actually able to organize pollen grains.

Out of the 16 pollen grains investigated here, only 4, out of the 7, numbers found at anaphase were actually found (Table 3, Fig. 8). Anaphase I numbers not found in pollen grains are 10, 12 and 21. However, the new numbers found in pollen grains are 15, 18 and 23. In addition, micropollen grains with only 1 chromosome were also found (Fig. 9). The discrepancy between anaphase and pollen grain mitosis is due to the smaller number of cells sampled.

Table 3. Karyotypic analysis of pollen grains

Chromosome number	Chromosome type										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
<i>Micropollen</i>											
1	—	—	—	—	—	—	—	1	—	—	—
1	—	—	—	—	—	—	—	1	—	—	—
1	—	—	—	—	—	—	—	—	—	1	—
1	—	—	—	—	—	—	—	—	—	—	1
<i>Macropollen</i>											
11	1	1	1	1	1	1	1	—	—	2	2
	1	1	1	1	1	1	1	1	1	1	1
13	2	1	1	2	1	1	1	1	1	1	1
14	3	1	1	1	1	1	1	1	1	1	2
	1	1	2	1	1	1	1	1	2	1	2
	2	1	2	1	1	—	1	2	1	2	1
15	1	2	2	1	1	2	1	1	1	1	2
	—	2	2	1	1	1	1	2	1	2	2
17+R	1	1	1	2	1	1	1	2	1	3	3
18	1	1	2	1	1	1	2	3	2	2	2
	1	2	1	1	2	2	1	1	1	3	3
23	2	2	3	2	2	1	3	2	2	1	3

A karyomorphological study of the pollen grain mitosis was also undertaken. The data is presented in Table 3. It is apparent that the grain with a chromosome number of 11 and above contain, except in three cases, at least one complete basic genome. In grains containing more than 11 chromosomes, the extra chromosome(s) may belong to any chromosome set and no preference is visible in this regard. Besides the 17 chromosomes in one pollen grain, an acentric ring was also found (Fig. 8). This might have arisen because of an interchange within a chromosome with the loss of the small segment having no centromere (Darlington 1965).

The micropollen containing a single chromosome (Fig. 9) is formed from lagging chromosomes. An analysis of all the four micropollen grains shows that their chromosomes belong to the short category (Types VIII, X and XI, see Fig. 11).

The number of micronuclei in mature pollen grains is less than expected

Figs. 6-9. 6, early telophase I with precociously dividing univalents. Note misdivision in one univalent. 7, telophase II showing one misdividing chromatid and bridge without fragment. 8, pollen grain mitosis I with 17+1 acentric ring. 9, micropollen grain with one chromosome. 10, pollen grain anaphase I with 16:17 segregation. Figs. 6-8 and 10, $\times 1,000$. Fig. 9, $\times 2,250$.

which may be due to the absorption of micronuclei by the cytoplasm (John and Lewis 1965). Maximum number of micronuclei in a pollen grain was 9 their size depends upon the size of the chromosome from which they arose.

Three pollen grain anaphases revealed a distribution of 12:12, 13:13 and 17:16 (Fig. 10). Some pollen grains showed intense fragmentation of chromosomes.

Discussion

From the above it is clear that in the male germ line rigorous selection for the complete genome of 33 chromosomes seems to be operative. As indicated earlier, the karyotype resolves into 11 types of 3 chromosomes (Fig. 11).

Fig. 11. Correlation of mitotic ($2n=33$) and meiotic ($11n+11n$) chromosomes and chromosome length and number of chiasmata.

There are, however, only two triplets in which the chromosomes do not resemble one another. One of them involves the long chromosome in which cases two chromosomes have equal long arms but unequal short arms. However, the third member of this triplet does not resemble them in either of the arms. The other is the nucleolar triplet in which only one member bears a secondary constriction. Both these points do not indicate auto-ploidy, but it seems hybridity may have been involved in the

origin of this taxon (Raina and Khoshoo 1971a,b). There are no other morphological characteristics in the karyotype which help in the diagnosis of the nature of its polyploidy. As shown by Stebbins (1950) karyotypic resem-

blances may be an unreliable criterion for diagnosing the nature of polyploidy because karyotype may not be entirely homologous even though they may be externally similar. Studies on *Paeonia* (Stebbins 1938), *Taraxacum* (Poddubnaja-Arnoldi 1939) and many other genera show that externally identical chromosomes may differ largely in structural rearrangements as revealed during meiosis. Like the present triploid *Crinum*, recently Southern (1967) found tetraploid tulip species were largely autopolyploid on the basis of karyotypic analysis, but their meiotic behaviour was indicative of allopolyploidy.

The heterozygous triplets involving long and nucleolar chromosomes offer the only markers indicating possible allopolyploid nature of *C. augustum*. The presence of only one nucleolar chromosome is perhaps the result of amphiplasty (Navashin 1934) and at least two genomes involving a strong and a weak nucleolar organizer constitute the triploid, the former suppressing the latter.

The metaphase I is characterized by a very low number (0.46) and range (0-2) of trivalents but a very high number of bivalents (10.51) and univalents (10.6) (Figs. 1-2, Table 1). The most common configuration seen is 11 bivalents and 11 univalents (Fig. 2). As is apparent from the Fig. 11 the two haploid sets of one genome form bivalents and remaining set is left unpaired. This means that the taxon is of hybrid constitution and could have arisen from the two distinct but related species. The first such hybrid triploid described by Rosenberg (1909) between *Drosera longifolia* ($n=20$) with *D. rotundifolia* ($n=10$) did not possess trivalents but 10 bivalents and 10 univalents. Such *Drosera* scheme is found in a number of other triploid hybrids involving 2 similar and one dissimilar genomes.

In many autotriploids, there is a low frequency of trivalents because of the small chromosome size and/or localization of chiasma formation. However, in *C. augustum* chromosomes are long and the distribution of chiasmata is at random. The low frequency of trivalents indicates a small measure of intergenomal pairing possibly because of small homologous segments. The trivalents are loosely attached and more often the third chromosome is attached by its end, or sometimes even by an attenuated chromatic thread. Perhaps a small homologous segment may be situated in the terminal regions but chromosomes are otherwise differentiated.

Anaphase I is characterized by unequal distribution precocious division and misdivision of univalents and bridges with or without fragments. The presence of bridges without fragments indicate pairing between chromosomes that are not completely homologous and thus there is a delay in disjunction (Darlington 1938, Akerman and Hagberg 1954, Murthy and Swaminathan 1956). The presence of bridge-fragment configurations both at anaphase I and anaphase II depends upon the number and position of chiasmata within a paracentric inversion (Swanson 1957). At any rate, it shows that the pairing chromosomes are heterozygous.

It emerges from the above that the marker chromosomes like the long and nucleolar chromosomes, in the karyotype of this triploid taxon, are indicative of hybridity having been involved in the origin of this taxon and that on karyotypic grounds the taxon is allo- or segmental allotriploid rather than autotriploid in constitution. This is in line with the predominant presence of 11 bivalents and 11 univalents during meiosis. Furthermore, the 11 univalents act rather independently in about 36.3% cells at anaphase I in that they divide. They seem to be differentiated from the chromosomes forming bivalents and follow their own regime. All these properties are indicative of a genomic formula of AAB or A_1A_2B . Such a conclusion corroborates the view that *C. augustum* is a taxon of hybrid origin from *C. bracteatum* $\times$ *C. zeylanicum* (see Schmidhauser 1954, Table 5).

The earlier reports about the nature of triploidy in *Crinum* were based on an analysis of mitotic chromosomes. Sato (1938) believed *C. macrantherum* to be autotriploid because of the 3 exactly similar sets in this species. Bose (1965) was not able to confirm this because of the non-availability of meiotic data.

In *C. augustum* besides macropollen (75.3μ) there is occurrence of 10% micropollen (7.3μ). The two types of pollen resemble in their exine and intine structure. Nearly 30% of the micropollen grains contain only one chromosome (Fig. 9) and appear to have arisen from the lagging chromosomes and rarely even chromosome fragments were also able to form such grains. The macropollen grains contained chromosome numbers $n=11, 13, 14, 15, 17, 18$ and 23.

Like most triploids due to irregular meiosis fertile pollen grains were of rare occurrence (1.0%) and there was no seed set. However, sexual reproduction is unnecessary for its survival because it is able to maintain its triploidy and consequent taxonomic individuality by vegetative reproduction. Many triploid species and cultivars have been reported in vegetatively reproduced taxa, such as *Iris*, *Rubus*, *Rosa*, *Hyacinth*, *Narcissus*, *Canna*, *Hemerocallis*, *Tulipa* and a number of other cases. *C. augustum* seems to have been preserved in cultivation, because of its large and showy flowers which are the result of combination of heterotic vigour due to hybridity and gigantism due to polyploidy.

Summary

Only cells with the full complement ($2n=33$) selected in the germ line during male meiosis. The average association of chromosomes at metaphase I is $0.46_{III}+10.5_{II}+10.6_I$ per cell. The most common association is $11_{II}+11_I$. Anaphases show irregular segregation, bridges with and without fragments, laggards, precocious division and misdivision. Pollen fertility is hardly 1%. Micropollen grains formed from the lagging univalents contained only one chromosome of the short category. The taxon is totally seed sterile but is

perpetuated by vegetative reproduction.

From the heteromorphicity of the longest triplet and the presence of only one nucleolar chromosome, it appears that the taxon is allo- or segmental allo-triploid which is supported by the presence of $11_{II}+11_I$ during meiosis.

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